from said active site residue is identified in said parent alpha-amylase structure and in new claims 91-92, the substrate binding area is identified in said parent alpha-amylase structure. New claims 87-92 are supported by the specification; no new matter has been added.

1. Claim Objections

Claim 85 has been objected to since it does not end with a period. In response, Applicant notes that claim 85 has been canceled.

It is asserted that claims 84 and 86 are objected to because they are substantial duplicates of each other. In response, claim 84 has been canceled.

2. The Rejections Under 35 U.S.C. §112, First Paragraph

Claim 83 has been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. It is asserted that the claim recites the specific fragments of SEQ ID NO:2 such as 1-103, 206-305, 104-205 and 396-483. The Examiner explained that she was unable to locate adequate support in the specification for such fragments of SEQ ID NO:2. Therefore, in the Examiner's view, there is no indication that these specific fragments were within the scope of the invention as conceived by Applicants at the time the application was filed.

First, Applicants note that claim 83 has been amended to recite that the A domain has an amino acid sequence corresponding to residues 1-103 and 206-395 of SEQ ID NO:2; said B domain has an amino acid sequence corresponding to residues 104-205 of SEQ ID NO:2 and said C domain has an amino acid sequence corresponding to residues 396-483 of SEQ ID NO:2. Amended claim 83 is supported by the specification on page 9, lines 1-6. Specifically, it is stated

> The domains can be defined as being residues 1-103 and 206-395 for domain A, residues 104-205 for domain B, and residues 396-483 for domain C, the number referring to the

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> *B licheniformis* α-amylase. This gives rise to an elongated molecule, the longest axis being about 85Å. The widest point perpendicular to this axis is approximately 50 Å and spans the central A domain. The active site residues of the *B. licheniformis* α-amylase **(SEQ ID NO 2)** are D323, D231 and E261.

In view of the amendment of claim 83 and the above arguments, Applicants assert that the rejection under 35 U.S.C. §112, first paragraph (written description) has been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

3. The Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 81-86 have been rejected under 35 U.S.C §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Each point raised by the Examiner will be addressed below.

A. Claim 81

It is asserted that claim 81 recites as step (a) "generating a three dimensional model of an alpha amylase". It is asserted that the claim is unclear because the generated three dimensional model should be of the parent alpha-amylase not any alpha-amylase. It is further asserted that it is unclear how the identifying is carried out in step (b). It is asserted that it can be done by comparing three dimensional structures of the parent amylase and the amylase of SEQ ID NO:13.

Applicants, in response, respectfully traverse the rejection. However, in order to advance prosecution, claim 81 has been amended to recite in step (a) that a three dimensional model of a parent alpha-amylase is generated. Furthermore step (b) has been amended to recite that at least one structural part or one amino acid of the parent alpha-amylase is identified.

Applicants further note that methods for identifying are provided on page 12, line 17-24. Specifically, it is stated:

> The analysis or comparison performed in step i) of the method according to the first, second and third aspect,

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> respectively, of the invention may be performed by use of any suitable computer program capable of analyzing and/or comparing protein structures, e.g. the computer program Insight, available from Biosym Technologies, Inc. For instance, the basic principle of structure comparison is that the three-dimensional structures to be compared are superimposed on the basis of an alignment of secondary structure elements... and the parts differing between the structures can subsequently easily be identified from the superimposed structure....

This is further clarified in the paragraph bridging pages 12 and 13:

> In the present context the term "structural or functional considerations" is intended to indicate the modifications are made on the basis of an analysis of the relevant structure or structural part and its contemplated impact on the function of the enzyme. Thus, an analysis of the structures of the various α-amylases, which until now has been elucidated, optionally in combination with an analysis of the functional differences between these α-amylases, may be used for assigning certain properties of the α-amylases to certain parts of the α-amylase structure or to contemplate such relationship. For instance, differences in the pattern or structure of loops surrounding the active site may result in differences in access to the active site of the substrate and thus differences in substrate specificity and/or cleavage pattern.

Furthermore, specific examples of identifying an amino acid residue(s) or structural part which when altered results in an altered property are provided in Examples 2 and 3.

B. Claim 82

It is asserted that claim 82 is unclear because of the recitation of "modeling methods", since the metes and bounds of the term "modeling methods" are not clearly defined rendering the scope of the claim unascertainable.

Applicants respectfully traverse the rejection. It is well-established case law that the words of a claim cannot be read in a vacuum but rather must be read in light of the specification and what is known in the art. *In re Moore*, 169 USPQ 236 (CCPA 1971). First, Applicants note that various references to "modeling methods" are made throughout

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the specification and would therefore clearly define the metes and bounds of this term. Specifically, on page 11, lines 9-14, it is stated

> Because of the high homology between the various Termamyl-like α-amylases, the solved structure defined by the coordinates of Appendix 1 is believed to be representative for the structure of all Termamyl-like α-amylases. A model structure of other Termamyl-like α-amylases may easily be built on the basis of the coordinates given in Appendix 1 adapted to the α-amylase in question by use of an alignment between the respective amino acid sequences. The creation of a model structure is exemplified in Example 1.

As stated in the specification, a description of the creation of a model structure is provided in Example 1. Various computer programs used for generating such a model structure such as HOMOLOGY and INSIGHT are disclosed. Applicants attach hereto as Exhibit 1 a description of the INSIGHT program by the vendor.

Furthermore, it is Applicants position that one of ordinary skill in the art would easily be able to define the metes and bounds of "modeling methods", since molecular modeling computer hardware and software are well known tools in the art which can be employed to generate a three-dimensional model given the relevant crystallography data (atomic coordinate data described in Appendix 1). Such an ordinary skilled artisan would fully understand how to configure the appropriate hardware and software and how to obtain such a three-dimensional model. Additionally, many modeling programs were well know in the art and readily available to a person of ordinary skill in the art as of the priority date of the instant application. Examples include RASMOL, LUDI and GRASP.

C. Claim 86

It is asserted that claim 86 recites as step (a) "generating a three dimensional model of an alpha amylase". It is asserted that the claim is unclear because the generated three dimensional model should be of the parent alpha-amylase not any alpha-amylase. It is further asserted that it is unclear how the utilizing is carried out in step (b) in addition

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to using "modeling methods". Again, it is asserted that the metes and bounds of the term "modeling methods" are not defined rendering the scope of the claim unascertainable.

Applicants, in response, respectfully traverse the rejection. However, in order to advance prosecution, claim 86 has been amended to recite that in step (a) that a three dimensional model of a parent alpha-amylase is generated. Furthermore, step (b) has been amended to recite that at least one structural part or one amino acid of the parent alpha-amylase is identified. Again, it is Applicants position for the reasons stated above, that the metes and bounds of "modeling methods" are clearly defined.

Applicants also assert that methods are taught for utilizing the model generated for identifying at least one amino acid residue or at least one structural part; wherein an alteration of such an amino acid residue or one structural part is predicted to result in an altered property. Specifically, on page 11, lines 15-21, a description is provided as to how the model is used:

> The above identified structurally characteristic parts of the Termamyl-like α-amylase, (Ca-binding site, substrate binding site, loops, etc.) may easily be identified in other Termamyl-like α-amylases on the basis of a model (or solved) structure of the relevant Termamyl-like α-amylase or simply on the basis of an alignment between the amino acid sequence of the Termamyl-like α-amylase in question with that of the *B. licheniformis* α-amylase used herein for identifying the amino acid residues of the respective structural elements.

The paragraph bridging pages 12 and 13 provide a further explanation as to how the model is utilized. This paragraph was provided earlier in the response (see top of page 8, *infra*). Specific applications of the model generated in Example 1 are disclosed in Example 2, with respect to determination of residues within 10Å from the ions present in the solved structure and in Example 3, with respect to determination of cavities in the solved structure.

D. Claims 83 and 85

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Claims 83 and 85 have been rejected as dependent from the rejected base claims. As noted above, claim 85 has been canceled and arguments have been made with respect to claim 81.

In view of the above arguments, Applicants assert that the rejections under 35 U.S.C. §112, second paragraph has been overcome. Therefore, Applicants respectfully request that the rejections be withdrawn.

4. Double Patenting Rejection

Claims 81-86 have been rejected under the judicially created doctrine of double patenting over claims 1-23 of U.S. Patent No. 5,989,169. In response, Applicants will address this issue upon indication of allowable subject matter.

5. Conclusions

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone at (914) 712-0093 if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: 3/4/02

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MARKED UP CLAIMS

81. (amended) A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase, wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca^{2+}-dependency and specific activity, wherein said parent alpha-amylase has a sequence of at least 70% homology to the sequence of SEQ ID No: 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.3) using default values for GAP penalties, said method comprising

(a) generating a three dimensional model of ~~an~~ a parent alpha-amylase structure, utilizing data from Appendix 1 and a computer programmed for generating said model from said data;

(b) identifying in said three-dimensional parent alpha-amylase structure generated in step (a) at least one amino acid residue or at least one structural part; wherein an alteration in said at least one amino acid residue or said at least one structural part is predicted to result in an altered property, and wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca^{2+}-dependency and specific activity;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue or at least one structural part identified instep (b); and

(d) expressing the modified nucleic acid of step (c) in a host cell to produce said variant alpha amylase.

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83. (amended) The method according to claim 81, wherein said three-dimensional alpha amylase structure has an A domain, a B domain and a C domain, wherein said A domain has an amino acid sequence corresponding to residues 1-103 and 206-~~305~~-395 of SEQ ID NO:2; said B domain has an amino acid sequence corresponding to residues 104-205 of SEQ ID ~~No.~~NO:2 and said C domain has an amino acid sequence corresponding to residues 396-483 of SEQ ID ~~No.~~NO:2.

86. (amended) A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase, wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca^{2+}-dependency and specific activity, wherein said parent alpha-amylase has a sequence of at least 70% homology to the sequence of SEQ ID No: 13, when homology is determined by the GAP program (Genetic Computer Group, Version 7.3) using default values for GAP penalties, said method comprising

(a) generating a model of a three dimensional structure of ~~an~~ a parent alpha-amylase ~~structure~~ using a computer programmed for generating a model structure and atomic coordinates shown in Appendix 1;

(b) utilizing said model generated in step (a) and modeling methods to identify at least one amino acid residue or at least one structural part; wherein an alteration in said at least one amino acid residue or said at least one structural part is predicted to result in an altered property, and wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation, Ca^{2+}-dependency and specific activity;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue or at least one structural part identified in step (b); and

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(d) expressing the modified nucleic acid of step (c) in a host cell to produce said variant alpha amylase.